

Modelling Proteins and their Interactions in the PP2A Network

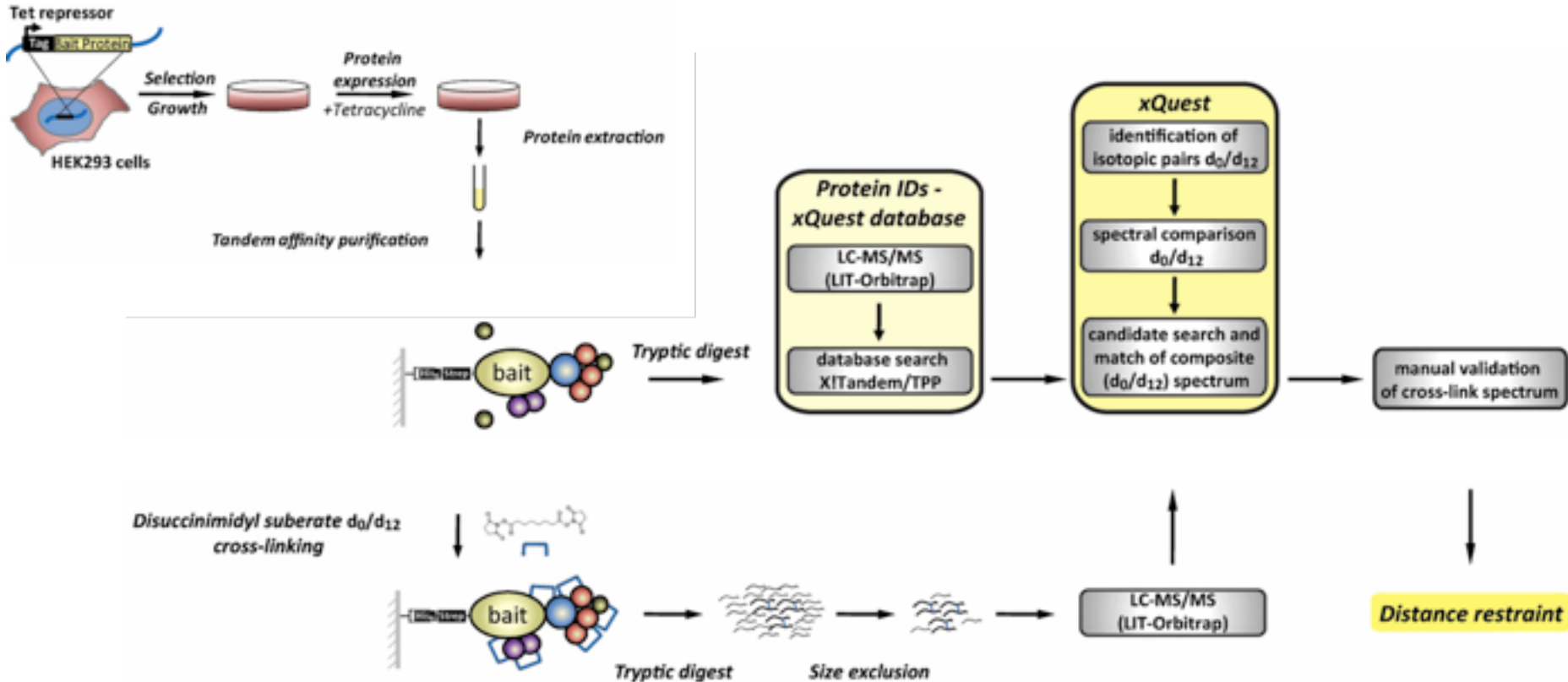
Using Cross-Link Data



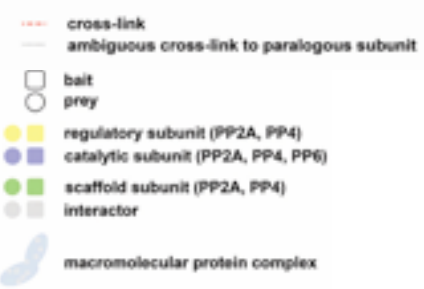
Abdullah Kahraman, PhD
Malmström Lab, ETH Zurich

RosettaCon 2012 - 30th July 2012

Affinity-Purification-Cross-Link-Mass-Spectrometry Workflow



Cross-Links (XL) on the PP2A Network

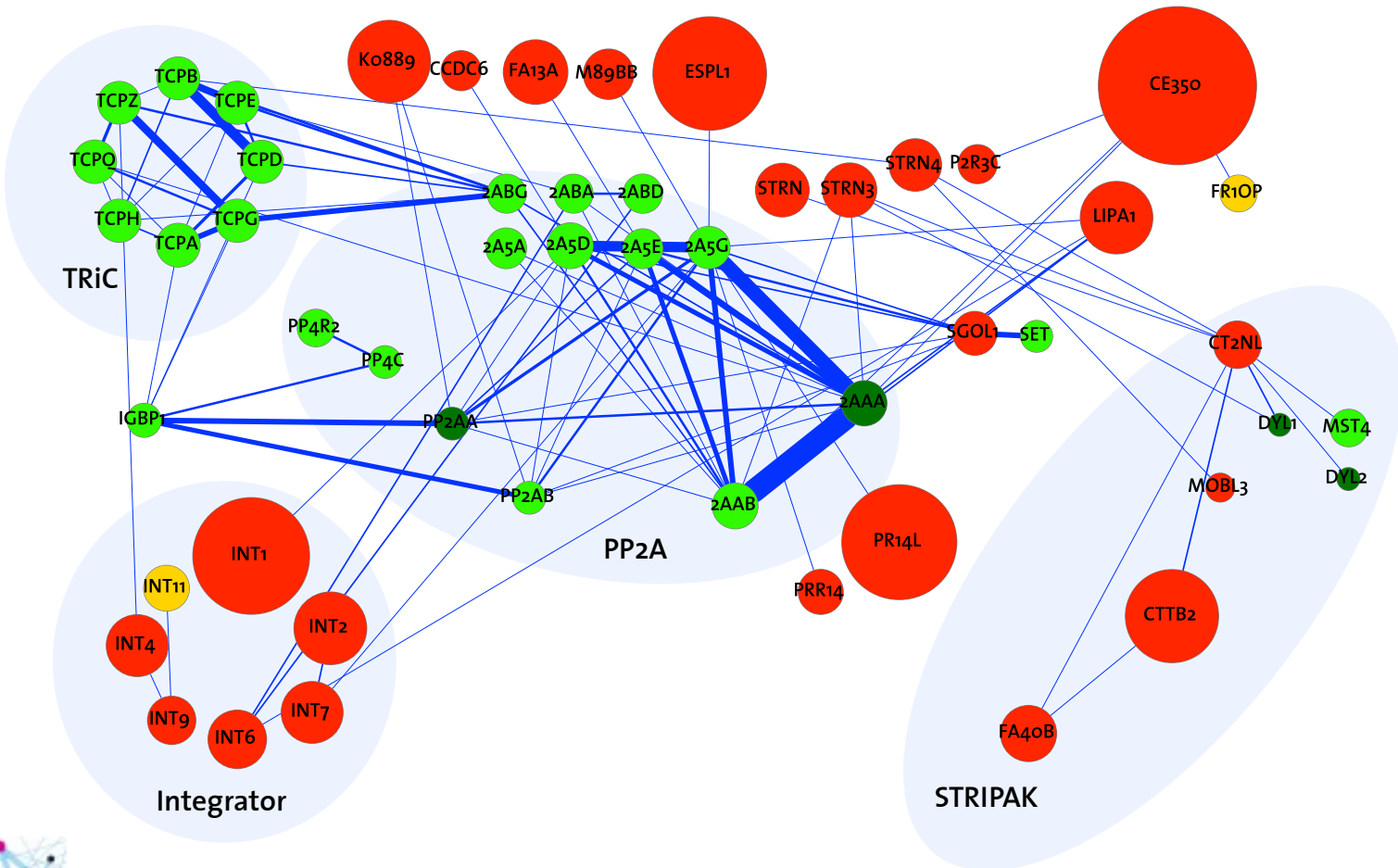


- 14 bait proteins
- 80 interactors
- 176 inter-protein XLs
- 570 intra-protein XLs



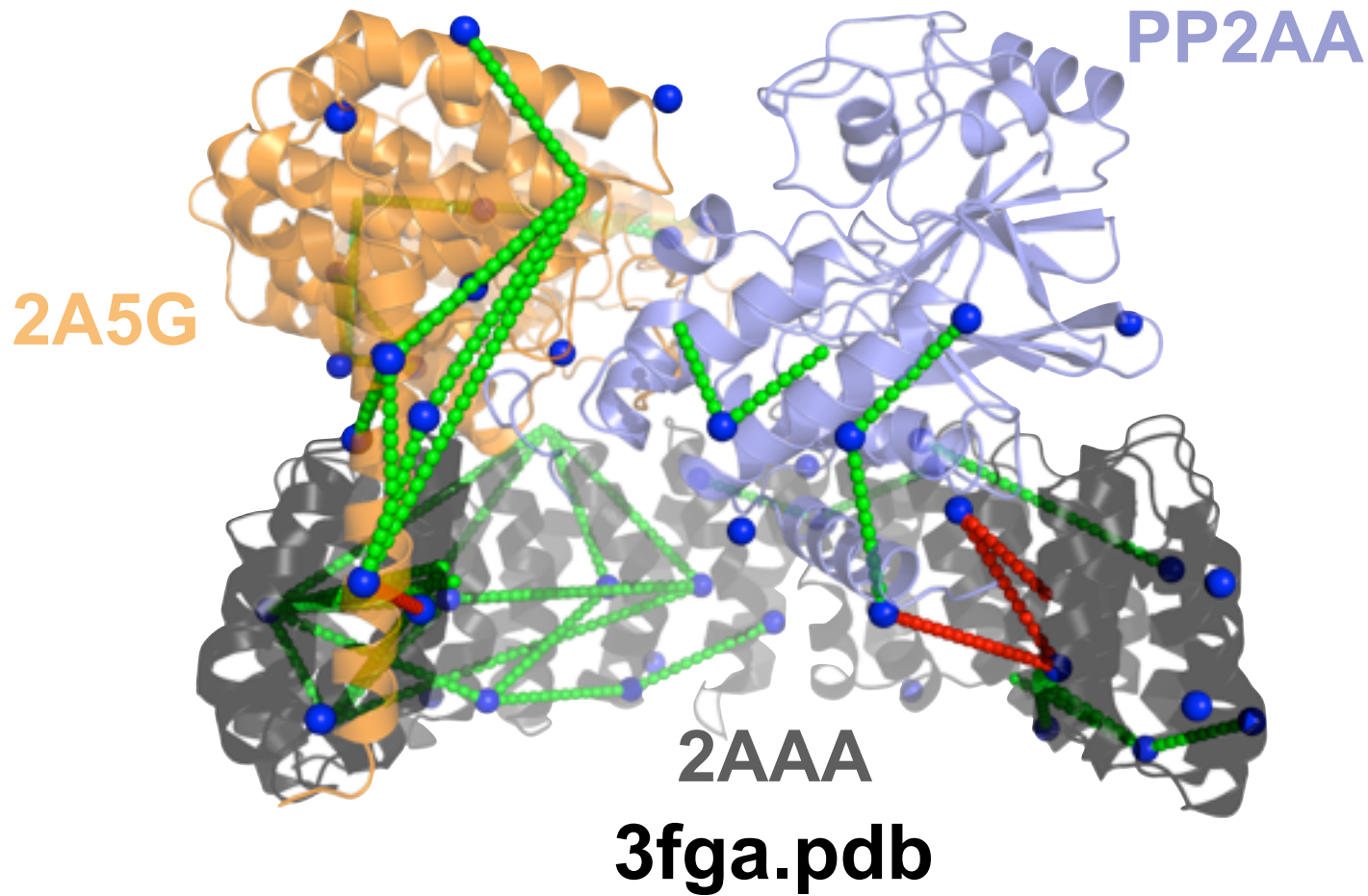
Structural Information on Network

- < 20% structural information ●
- < 40% structural information ●
- < 99% structural information ●
- X-ray structure ●
- Protein size ⊕
- Number of XLS —



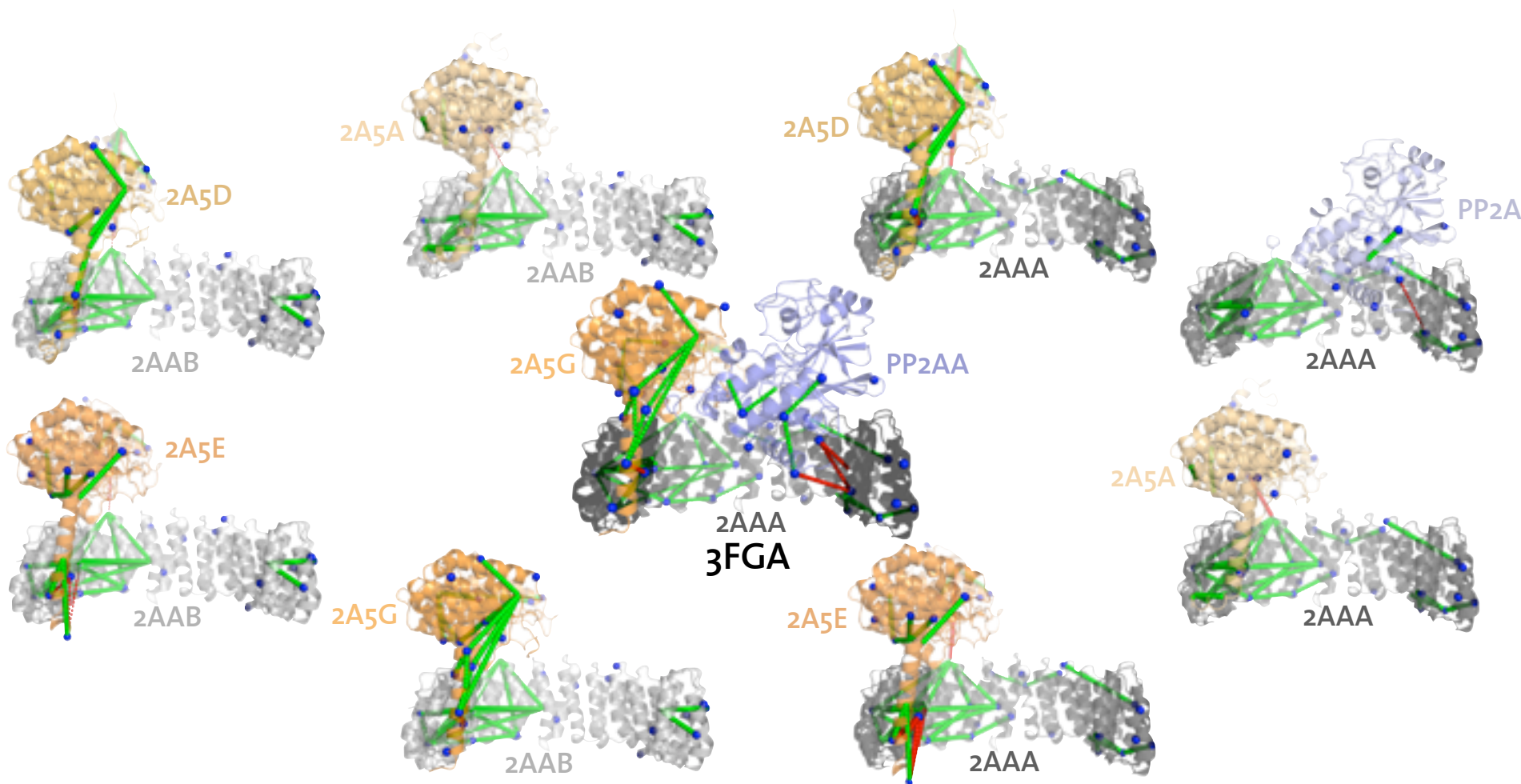
Validation of XLs on X-ray Structures

●●●●● intra-protein ●●●●● inter-protein ● mono-links



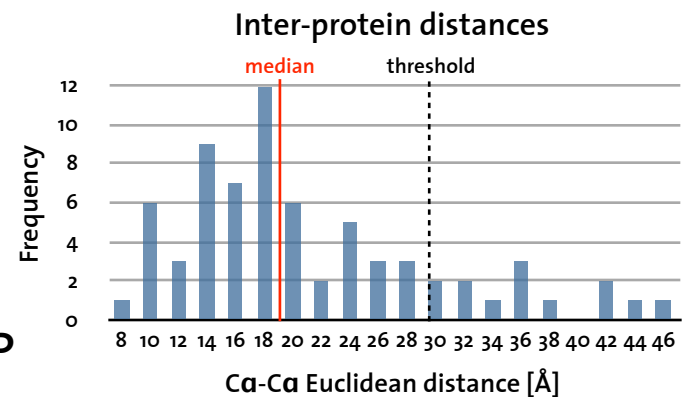
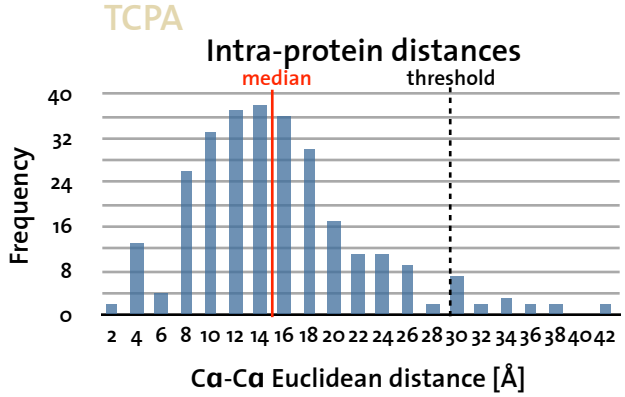
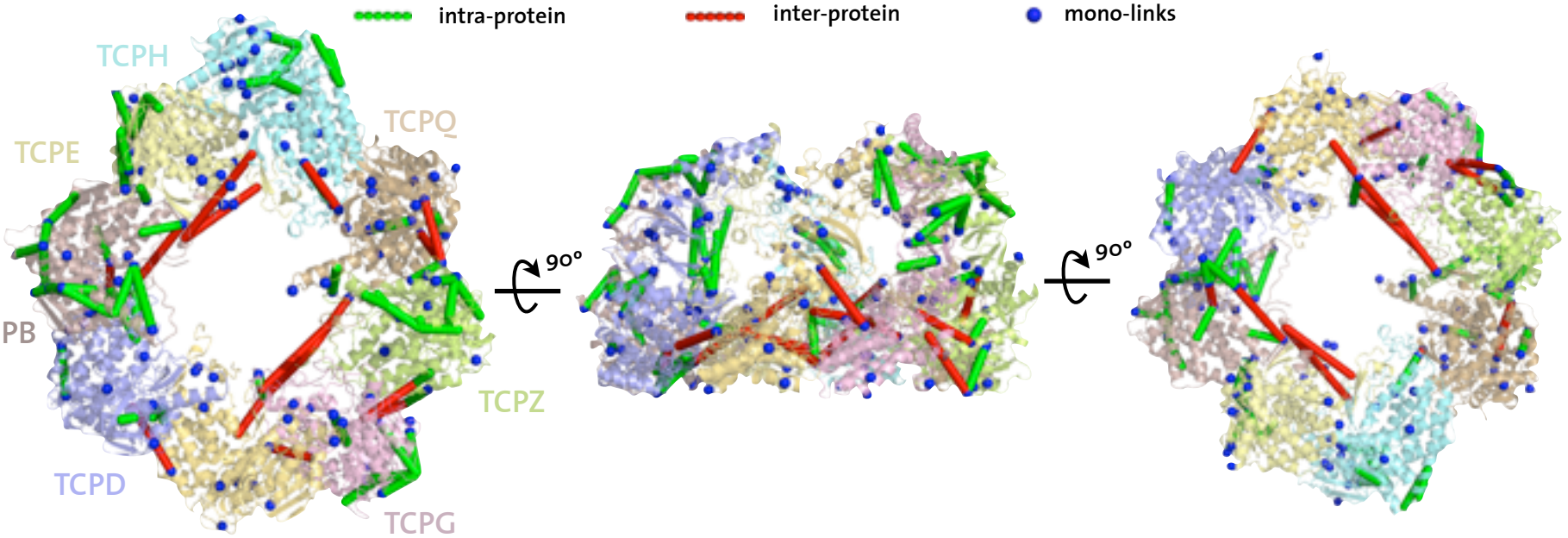
Validation on Comparative Models I

●●●●● intra-protein
 ●●●●● inter-protein
 ● mono-links



Thompson, J. & Baker, D. Incorporation of evolutionary information into Rosetta comparative modeling. *Proteins* **79**, 2380–2388 (2011).

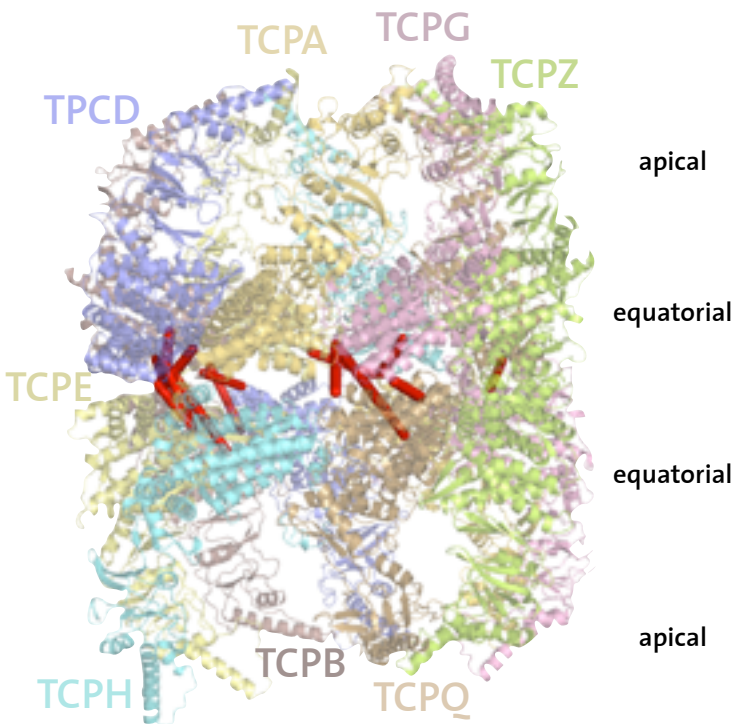
Validation on Comparative Models II



92% TP

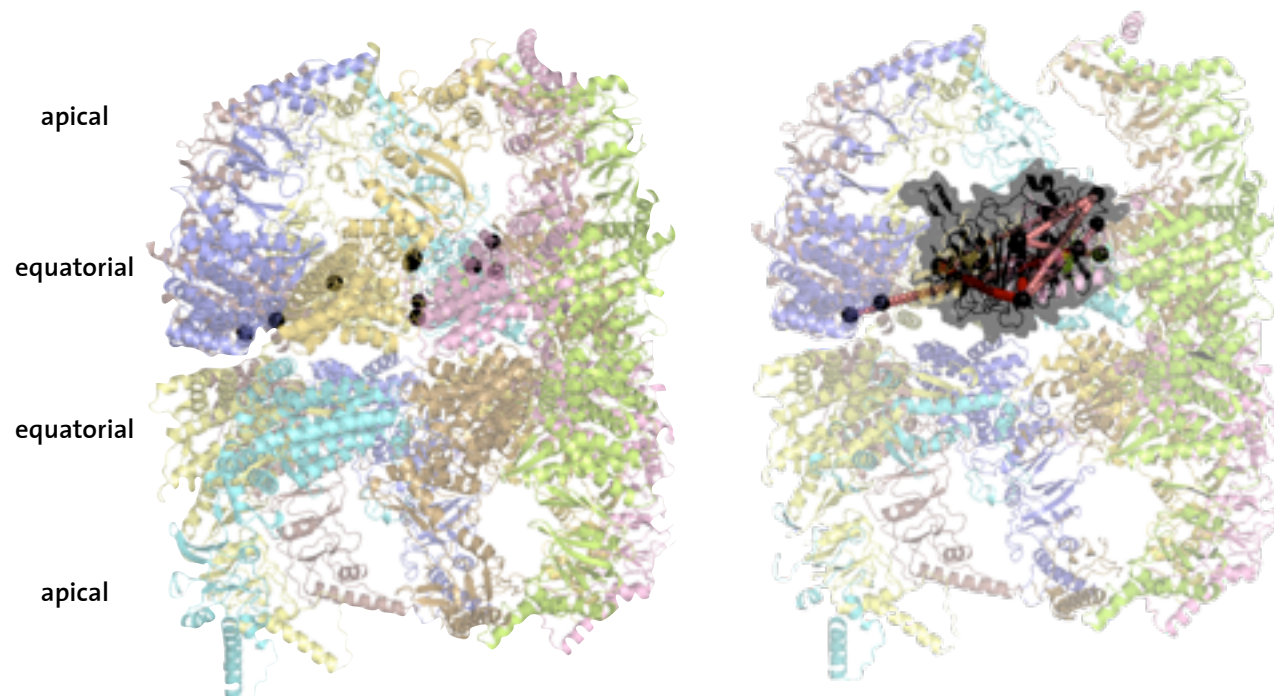
Munoz IG et al. (2012), Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. *Nat Struct Mol Biol* **18**, 14.
 Leitner A *et al.*, (2012) The Molecular Architecture of the Eukaryotic Chaperonin TRiC/CCT. *Structure*. Apr 11;

TRiC Chaperonin

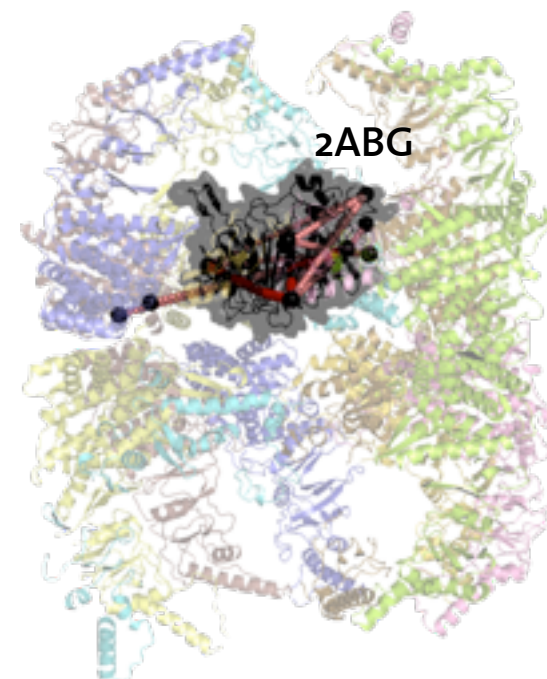
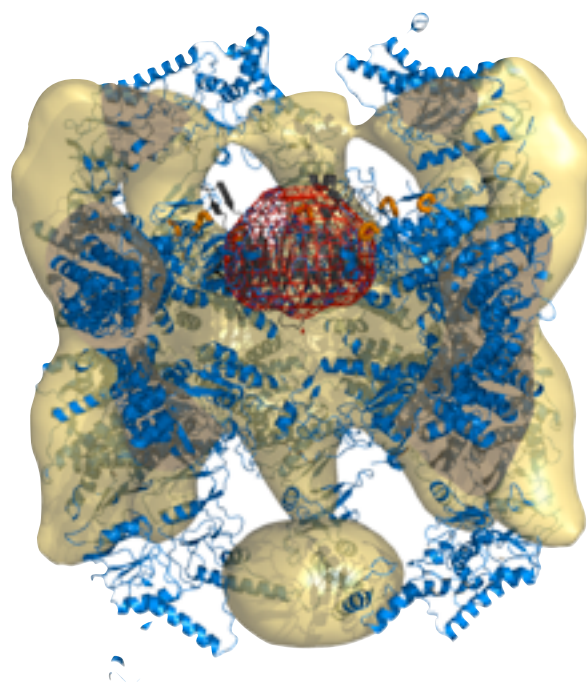


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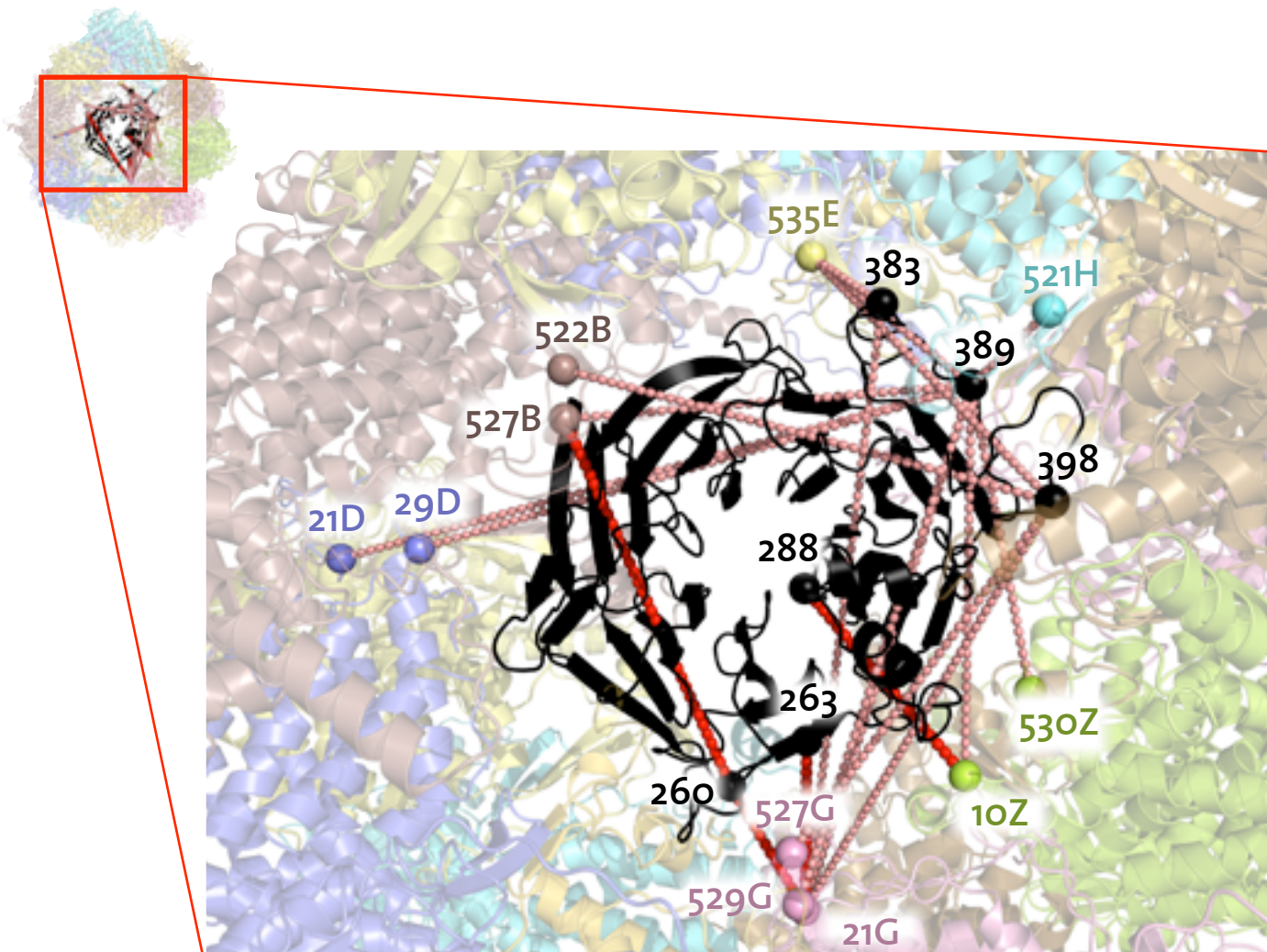
TRiC - 2ABG Interaction



TRiC - 2ABG Interaction



TRiC - 2ABG Interaction



XLs to a Partially Folded Intermediate?



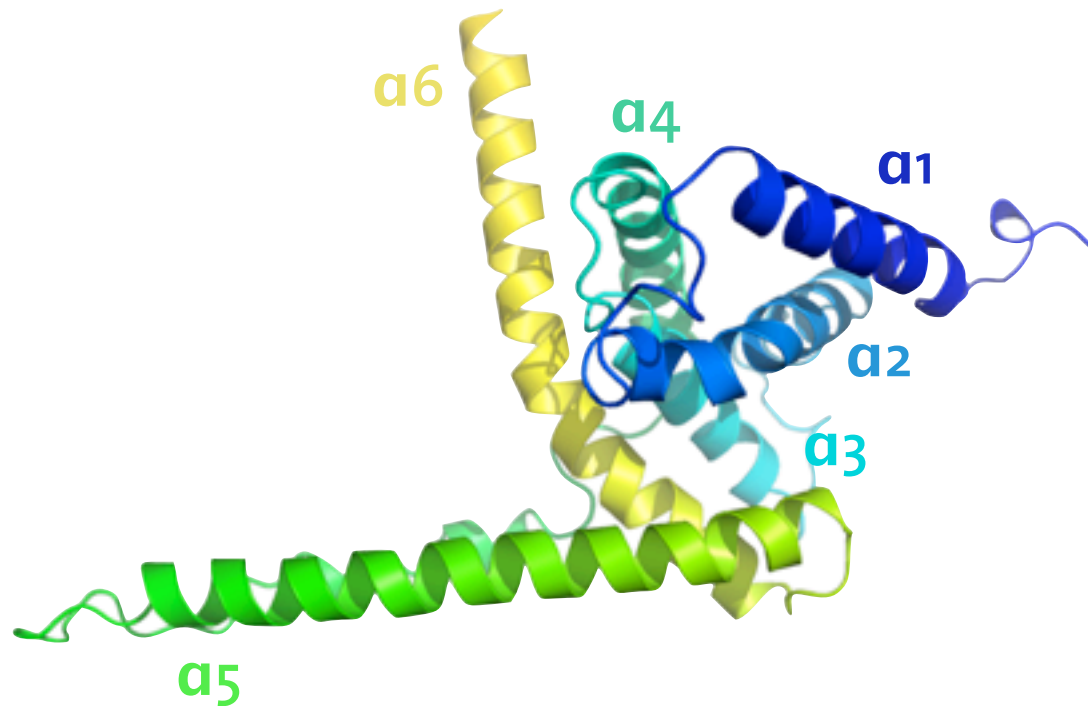
15 XLs conformed
19.5 Å RMSD to 2ABA



10 XLs conformed
2.2 Å RMSD to 2ABA



Full-length Model of IgBP1



Chris Miles, nonlocal protocol.

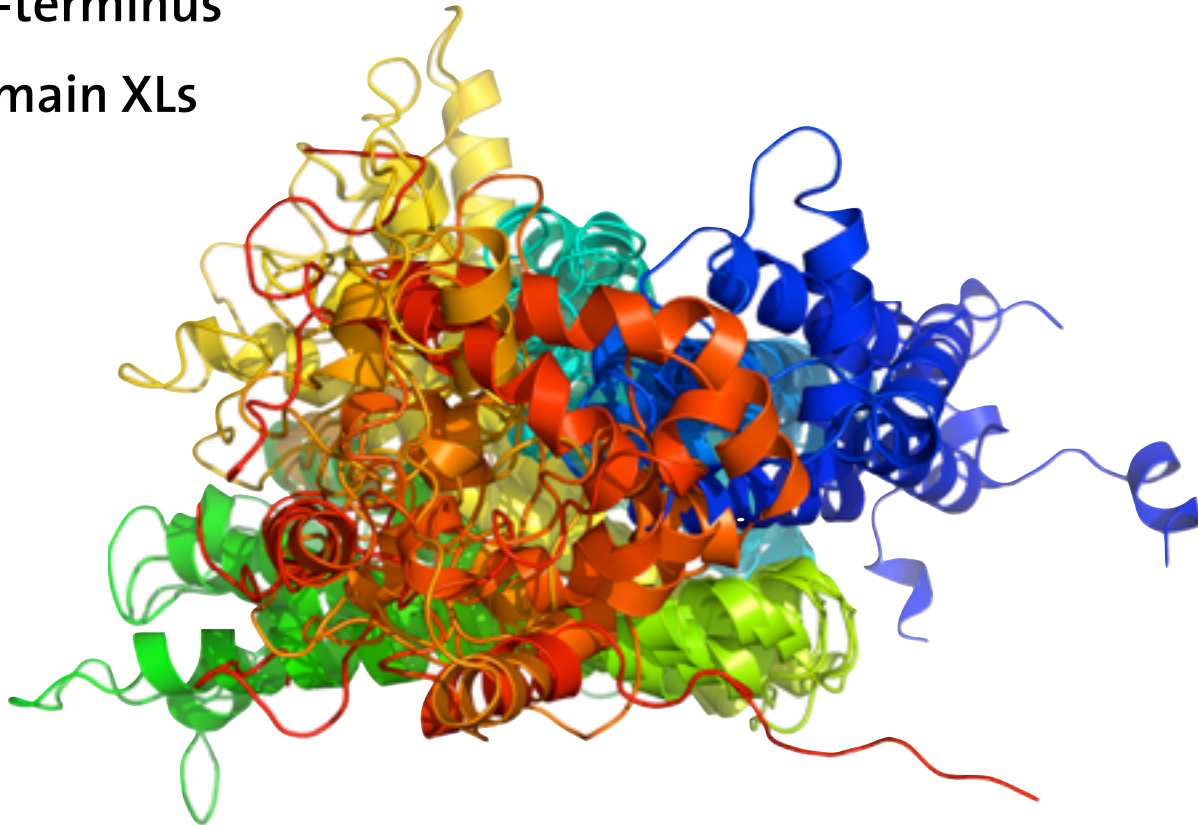
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Bradley, P. and Baker, D., Improved beta-protein structure prediction by multilevel optimization of nonlocal strand pairings and local backbone conformation.

Proteins: Structure, Function, and Bioinformatics, **65**: 922–929. (2006)

Full-length Model of IgBP1

- 60 intra-protein XLs conformed
- 18 within C-terminus
- 32 inter-domain XLs



Chris Miles, nonlocal protocol.

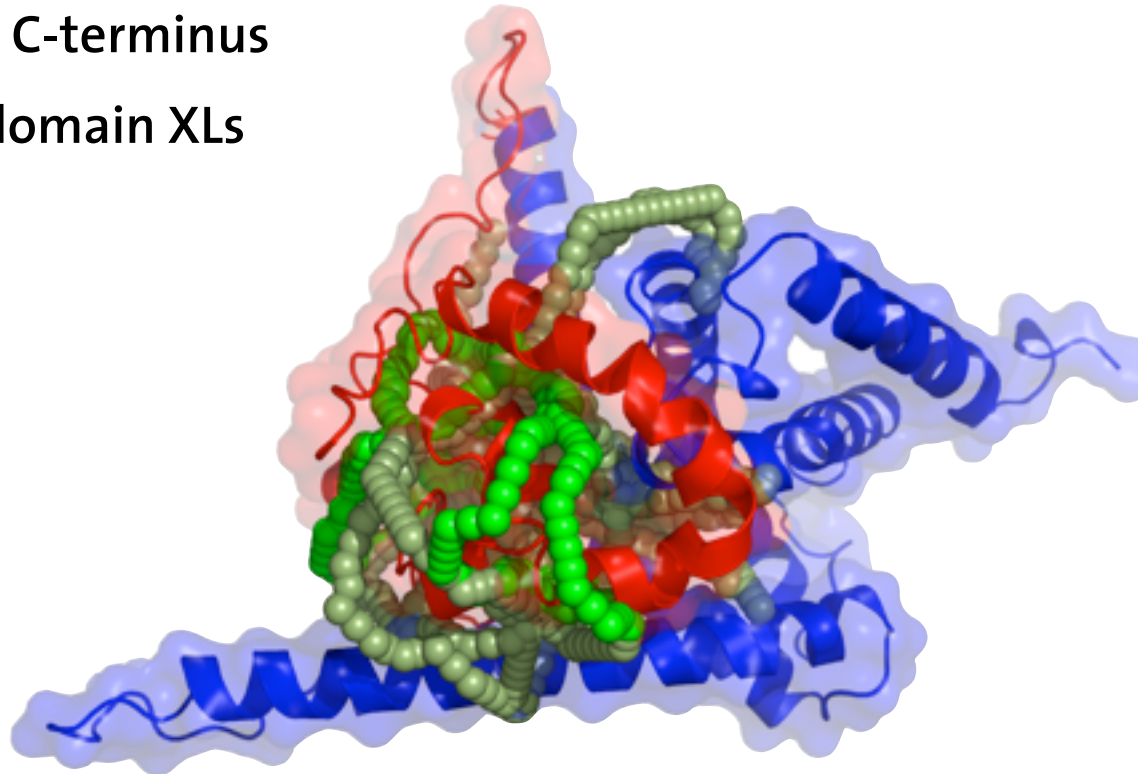
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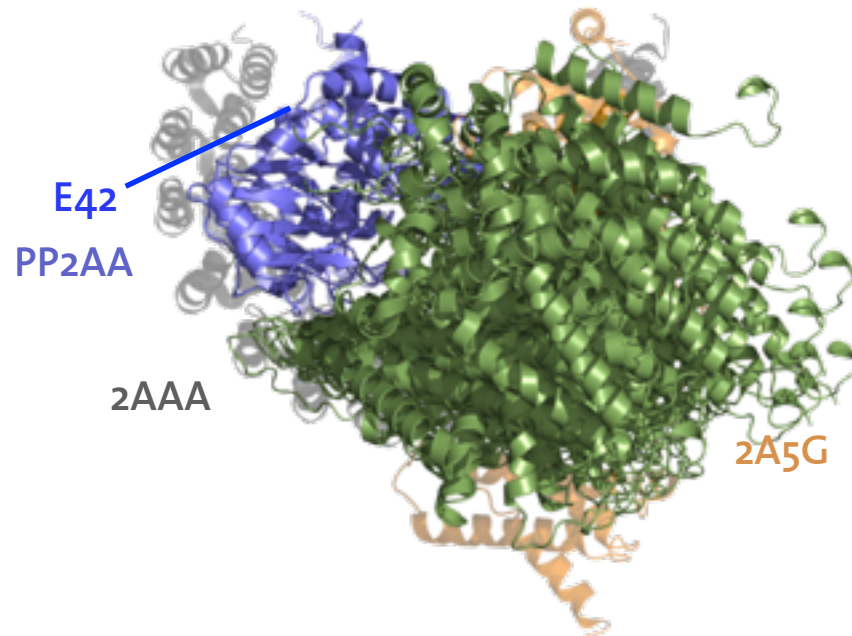
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XL Guided Docking

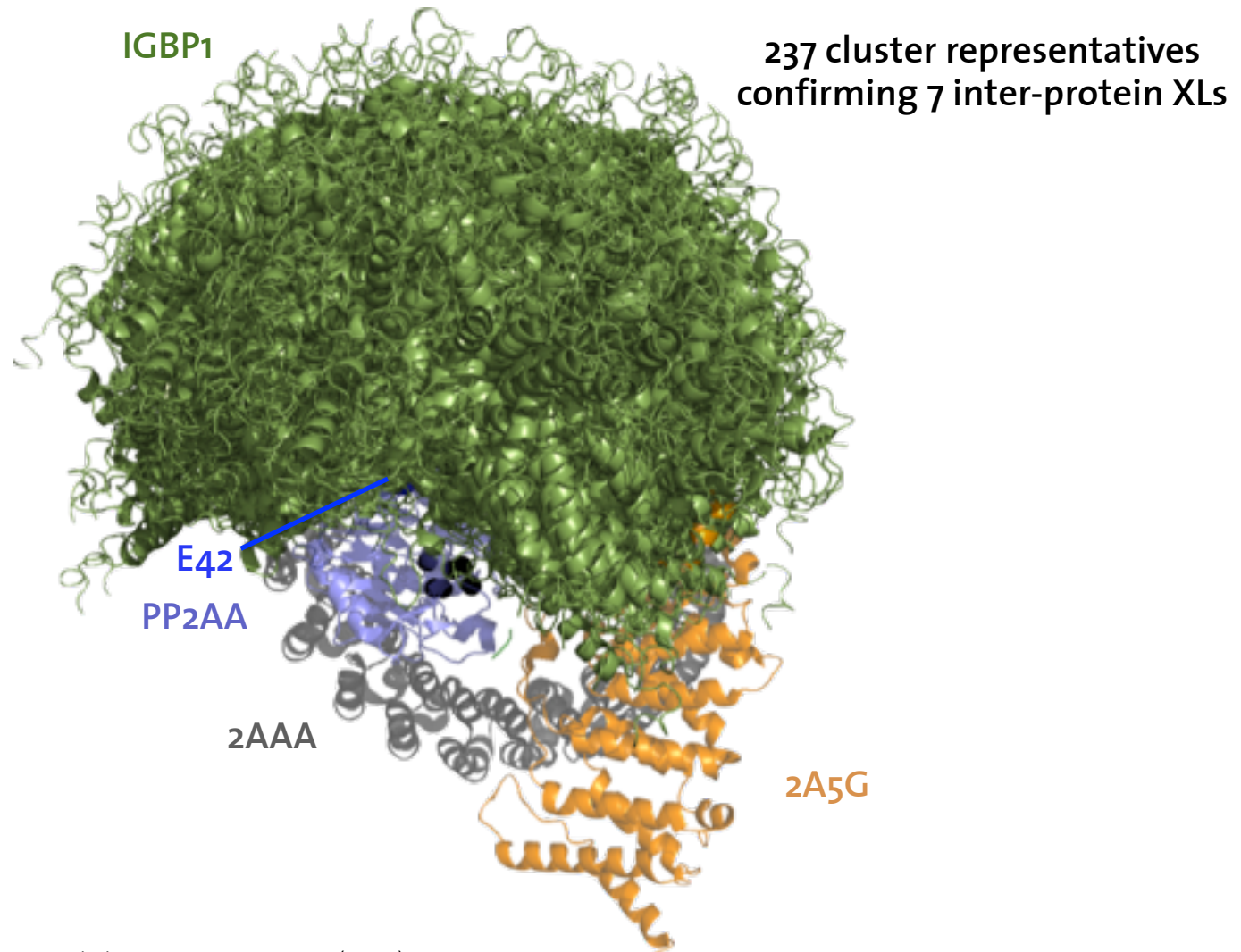
IGBP1

10 cluster representatives based on
ab initio unconstrained docking



Prickett, T. & Brautigam, D., *J Biol Chem* **279**, 38912–38920 (2004).

XL Guided Docking

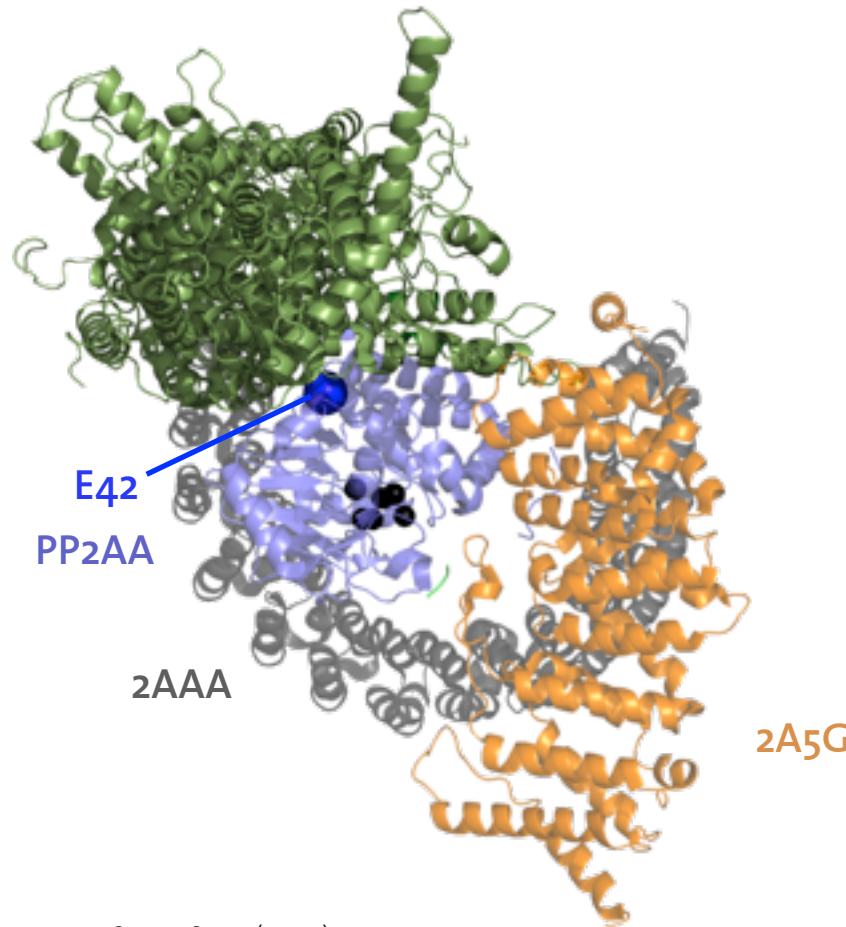


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XL Guided Docking

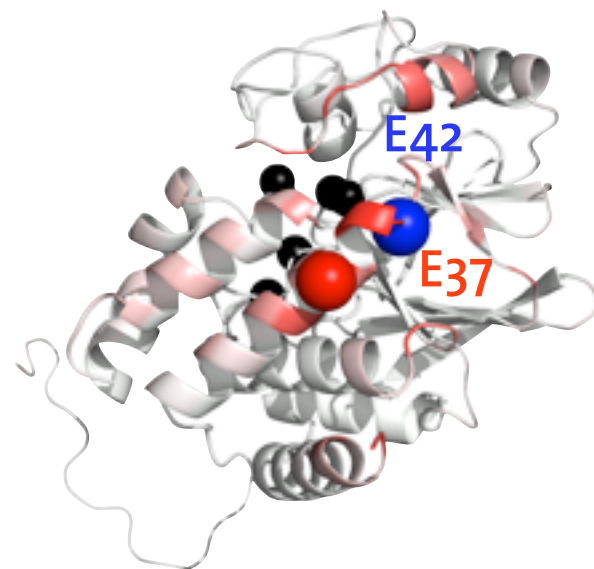
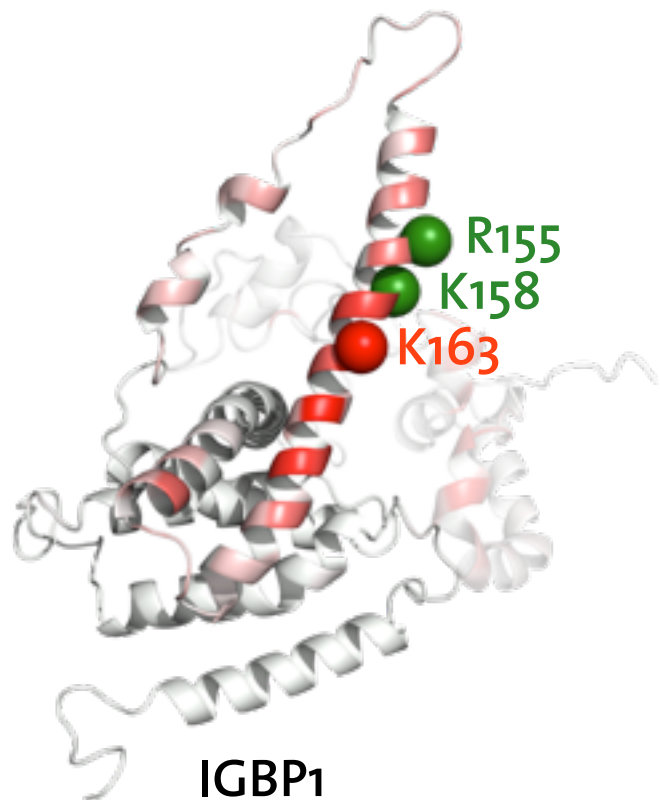
IGBP1

4 cluster representatives with
shortest average inter-protein SASD



Prickett, T. & Brautigam, D., *J Biol Chem* **279**, 38912–38920 (2004).

IGBP1-PP2AA Interface Prediction



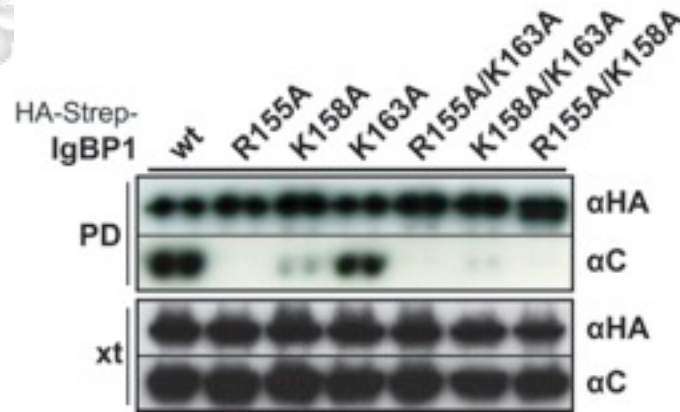
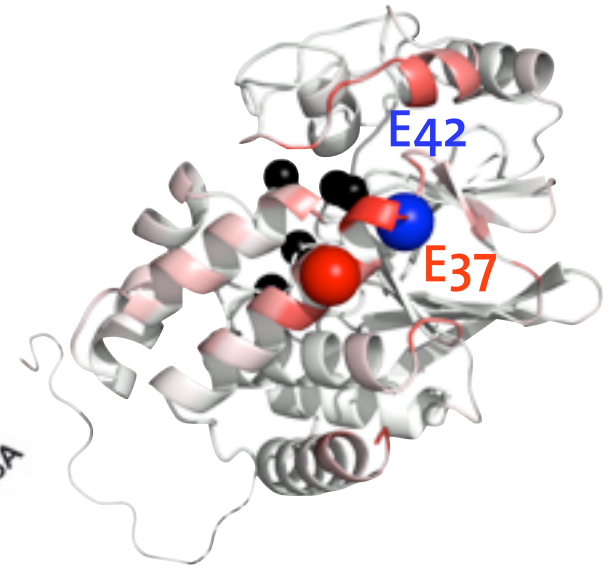
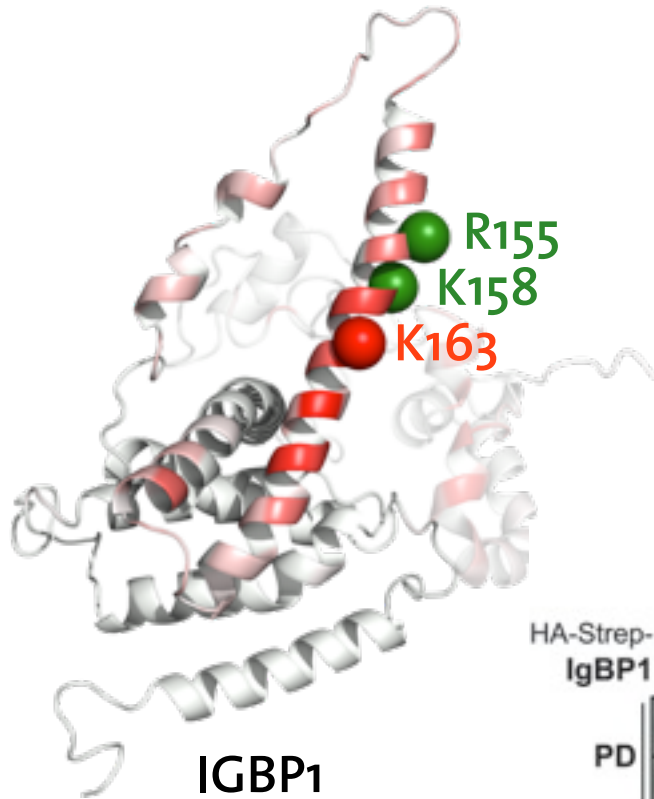
0% 100%

frequency at interface

Prickett, T. & Brautigan, D., *J Biol Chem* **279**, 38912–38920 (2004).

LeNoue-Newton, M. *et al.*, *J Biol Chem* **286**, 17665–17671 (2011).

IGBP1-PP2AA Interface Prediction



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Acknowledgement



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Herzog F*, Kahraman A*, Böhringer D, Mak R, Bracher A, Walzthoeni T, Leitner A, Beck M, Hartl FU, Ban N, Malmström L, Aebersold R (2012), Structural Probing of a Protein Phosphatase 2A Network by Chemical Cross-linking and Mass Spectrometry, *Science* (accepted)

